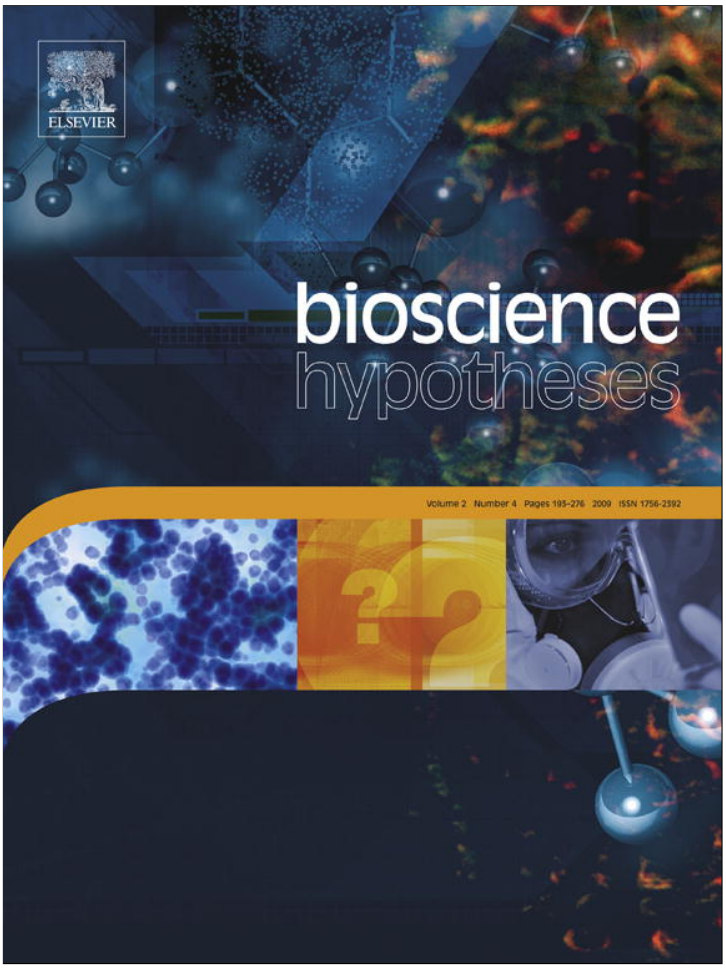

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Passive and active stabilization of dopamine in the striatum

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Abstract Parkinson's disease is a neurodegenerative disorder associated with cell loss from the substantia nigra pars compacta (SNc). The dopaminergic cells of the SNc project to the striatum where the loss of dopaminergic tone is thought to be the main cause of Parkinsonism symptoms. Animal models have shown that striatal tissue content of dopamine declines proportionally to cell death in the SNc but the extracellular concentration of dopamine (EDA) in the striatum remains near normal until more than 85% of SNc neurons have died. We investigate various explanations for the remarkable homeostasis of EDA with a mathematical model that has recently been constructed for dopamine synthesis, release, and reuptake, which includes the effects of the autoreceptors. We provide evidence and explanations for the passive stabilization hypothesis and show that the autoreceptors enhance stabilization of EDA only when fewer than 25% of the SNc cells remain.

Introduction

Parkinson's disease is a neurodegenerative disorder associated with cell loss from the substantia nigra pars compacta (SNc) [1]. The dopaminergic cells of the SNc send projections to the striatum where the loss of dopaminergic tone is thought to be the main cause of tremor and other motor symptoms of Parkinsonism [2,3]. A particularly interesting and important feature of the disease is that symptoms do not appear until a very large percentage (60–90%) of SNc cells have died and therefore this feature has been the focus of much experimental and clinical investigation [4,5]. Experiments with animal models [6–8] have shown that although striatal tissue content of dopamine declines more or less proportionally to cell death in the SNc, the extracellular concentration of dopamine (EDA) in the striatum remains near normal until more than 85% of SNc neurons have died. This is widely believed to be the reason that symptoms do not appear until very late in the degeneration of the SNc.

What is the basis of this remarkable homeostasis of striatal EDA in the face of progressive cell death in the SNc? Hornykiewicz [9] has proposed that the nigrostriatal system adapts to cell death to maintain EDA level. Zygmond [4,10]

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suggested that the remaining living SNc cells adapt by increasing dopamine (DA) synthesis and it is known that they sprout more terminals [11]. Garris and co-workers [8,12,13] have introduced the concept of ''passive stabilization,'' which explains the homeostatic effect by simple principles of release, reuptake, and diffusion, without the necessity of appealing to active adaptive mechanisms.

We have constructed a detailed mathematical model of dopamine synthesis and release [14]. Our purpose here is to use results from model simulations as a basis for discussing and clarifying the roles of different mechanisms in the homeostasis of EDA.

Passive stabilization

In their 2003 paper, Bergstrom and Garris give experimental evidence for passive stabilization and they explain clearly the reason for the effect [8]. The idea is that if only half the cells in the SNc remain alive, then only half as much dopamine will be released into the extracellular space per unit time. However, there will also be only half as many DA transporters (DATs) to remove EDA from the extracellular space so the steady state concentration of EDA should remain the same. They show, in fact, that both overall release and overall reuptake are (approximately) proportional to f, the fraction of cells still innervating the striatum. They do not, however, explain why the homeostatic effect breaks down when f is small, although they do suggest that it might be due to the diffusion gradients between the sparse remaining living terminals.

The solid curve in Fig. 1 shows the model EDA concentration as a function of f, the fraction of cells remaining alive, when the living cells have the ''normal'' number of DATs. Note that the EDA curve does not start to plunge toward zero until only 10% of the cells in the SNc are left. If one runs the same simulations but with only 1/4 as many DATs on each cell, then the EDA concentration is much less homeostatic as f gets smaller (long dashes). And if there is only 5% of the usual number of DATs, then the homeostatic effect disappears almost entirely (short dashes) and EDA declines almost proportionally to f. This clearly shows that the strength of passive stabilization depends on the number of DATs.

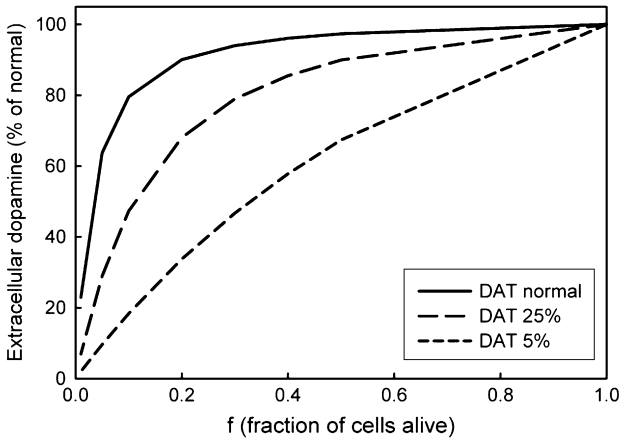

Figure 1 Passive stabilization depends on the DATs. The solid line shows the EDA concentration as a function of f, the fraction of SNc cells still alive, when the autoreceptors are turned off and the rate constant for diffusion out of the system is constant. If the number of DATs is reduced to 25% of normal, their homeostatic effect is smaller (long dashes) and if they are reduced to 5% (short dashes) the homeostatic effect almost vanishes. Computations were performed with the mathematical model in [14].

We note that in these simulations we are assuming that the extracellular space is well-mixed. That is, the DATs on each living neuron see an EDA concentration that is the average extracellular concentration over the whole extracellular space. As f gets very small, this assumption has less justification.

Removal from the system

If Garris' passive stabilization idea is correct, why doesn't it work for all f; and why doesn't EDA simply remain constant as f declines to zero? The reason is that dopamine is removed from the system by various mechanisms. Dopamine is removed from the extracellular space by catabolism, uptake into glial cells, uptake into the blood, and by diffusing out of the striatum. When Bergstrom and Garris say ''diffusion'' they mean diffusion from one synapse to another, what is usually called volume transmission or spillover [15,16]. This kind of diffusion is implicit in the ''well-mixed'' assumption referred to above and is included in our model because each neuron's DATs reuptake from the same extracellular pool of EDA. So, extracellular dopamine has two fates. It is reimported into terminals and it is removed from the system (via one of the mechanisms described above) at a rate k_{rem}[EDA] μM/h, where k_{rem} represents all of these removal mechanisms. Normally, the effect of this removal term in the mathematical model is very small, because most released dopamine is rapidly transported back into terminals (though not necessarily the same terminal) by the DATs [17,18]. However, as f declines and the amount of dopamine released and taken up by the DATs declines, this removal becomes relatively more important. That is why the solid curve in Fig. 1 declines to zero as as f goes to zero. In computing the curves in Fig. 1, k_{rem} was set to 400 μM/h (see [14]).

In fact, we believe that the removal coefficient k_{rem} should get larger as f gets smaller. We reason that in the normal case, the synapses of the dopaminergic neurons of the SNc in the striatum are very densely packed [19,20]. Thus it is very hard for a dopamine molecule released from one terminal to escape from the system without running into a DAT and being reimported into another terminal. However, after many SNc cells have died, the remaining terminals will be much more sparse and it will be much easier for any given dopamine molecule to find its way out of the system without encountering a DAT. This idea was also suggested in [8]. But, how should k_{rem} increase as f declines? Even given regular geometry this is an extremely difficult question mathematically, and it is probably not worth considering in detail until more detailed information about the geometry of synaptic terminals in the striatum becomes available. However, we experimented with two choices in the model. The dashed curve in Fig. 2 shows what happens to the passive stabilization curve (solid) in Fig. 1 if one assumes that k_{rem} is proportional to $1/f$. There is much

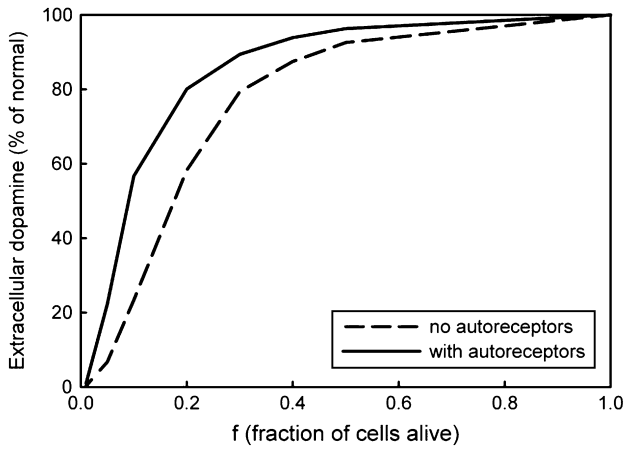

Figure 2 Removal of DA from the system ($k_{rem} = 1/f$) and autoreceptors affect stabilization. The EDA concentration is shown as a function of f if the normal number of DATs is present, the autoreceptors are turned off, and removal from the system is proportional to $1/f$ (dashed curve). If the autoreceptors are turned on, the EDA concentration becomes more homeostatic (solid curve).

less of a homeostatic effect. If k_{rem} is proportional to $1/\sqrt{f}$, that is, k_{rem} grows more slowly as f falls, then one obtains the dashed curve in Fig. 3. The homeostatic effect is better than the dashed curve in Fig. 2, but still not as good as the solid curve in Fig. 1 where there was no dependence of k_{rem} on f. Thus removal from the system decreases homeostasis of EDA, but only becomes an important effect when the lesion is quite large, i.e. f is quite small.

The autoreceptors

In computing all the curves in Fig. 1 and the dashed curves in Figs. 2 and 3, the autoreceptors in our model were turned off. It is known that the autoreceptors inhibit DA synthesis and release, and inhibit firing [21–23]. When we turn the autoreceptors on in our model, we get the solid curves in Fig. 2 or Fig. 3 depending on whether we assume that k_{rem} is proportional to $1/f$ or to $1/\sqrt{f}$. In both cases the autoreceptors increase the homeostatic effect. This is because the autoreceptors release their inhibition when the EDA concentration drops so that each remaining living terminal releases more dopamine into the extracellular space. Notice that this effect does not occur until f is quite low because as long as passive stabilization keeps the EDA concentration near normal, the autoreceptors do not release their inhibition. However, when the EDA concentration starts dropping significantly because of removal from the system, the autoreceptors kick in and stabilize the EDA concentration. This is confirmed by the experimental literature where increased synthesis and release are not seen until f is quite small [4,10,24]. Fig. 4 shows the velocity of the tyrosine hydroxylase reaction in the model as a function of f in the two cases, k_{rem} proportional to $1/f$ or to $1/\sqrt{f}$. As one can see, synthesis does not rise significantly until f is small.

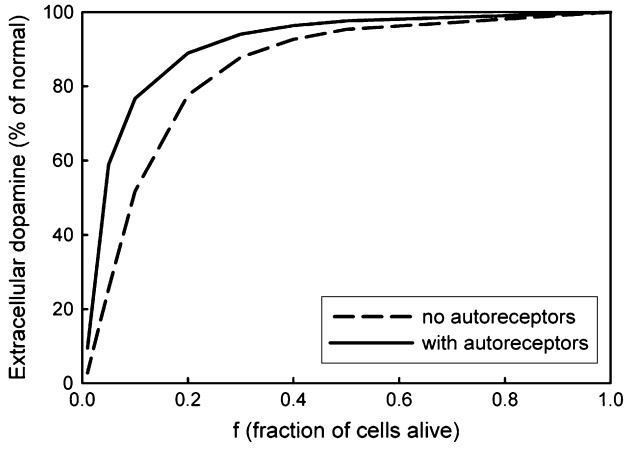

Figure 3 Removal of DA from the system ($k_{rem} = 1/\sqrt{f}$) and autoreceptors affect stabilization. The EDA concentration is shown as a function of f if the normal number of DATs is present, the autoreceptors are turned off, and removal from the system is proportional to $1/\sqrt{f}$ (dashed curve). If the autoreceptors are turned on, the EDA concentration becomes more homeostatic (solid curve).

A simple model

The results shown in Figs. 1–4 are based on computations with our detailed model [14] that includes the import of tyrosine, the synthesis of dopamine in the cytoplasm, storage in vesicles, release, reuptake by the DATs, and the influence of the autoreceptors. But, some of the ideas that we have discussed can also be understood by using the simple model for EDA introduced by Wightman and coworkers [25,26]:

$$\frac{dE}{dt} = R - \frac{V_{max}E(t)}{K_m + E(t)} \tag{1}$$

where $E(t)$ represents extracellular dopamine, R is the rate of release, and V_{max} and K_m are the parameters for the total reuptake of EDA back into the terminals by the DATs. For normal firing, either tonic or bursting, the EDA concentration is in the low nanomolar range [17] well below $K_m \approx 0.2\ \mu M$. Thus, for considering steady states we can rewrite Eq. (1) approximately as:

$$\frac{dE}{dt} = R - \frac{V_{max}}{K_m}E(t) \tag{2}$$

Setting the right hand side equal to zero we see that the steady state is $E_{ss} = R/(V_{max}/K_m)$ Now suppose that only the fraction f of SNc cells remain alive. Then, assuming that these cells operate as before, release R is replaced by fR and reuptake $(V_{max}/K_m)E$ is replaced by $f(V_{max}/K_m)E$ giving the equation

$$\frac{dE}{dt} = fR - f\frac{V_{max}}{K_m}E(t) \tag{3}$$

The steady state $E_{ss} = R/(V_{max}/K_m)$ remains the same as before because when one sets the right hand side of Eq. (3) to zero and solves for E_{ss}, the fs cancel out. This is the underlying reason for passive stabilization, clearly expressed in [8]. If this is true, why does passive stabilization fail as f becomes small? As explained above, what

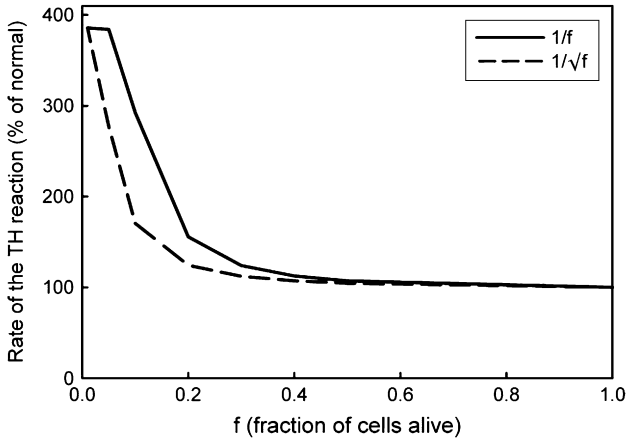

Figure 4 Synthesis of dopamine as a function of cell death. The curves show the velocity of the TH reaction in the case $k_{rem} = 1/f$ (solid line) and the case $k_{rem} = 1/\sqrt{f}$ (dashed line). In both cases, synthesis does not rise significantly until f is small.

has been neglected is removal of dopamine from the system by diffusion out of the striatum, uptake by glial cells and the blood, and catabolism in the extracellular space. To represent all these complicated processes in the Wightman model, we add a removal term

$$\frac{dE}{dt} = fR - f\frac{V_{max}}{K_m}E(t) - uE(t) \quad (4)$$

where u gives the rate of removal per unit time. Now the steady state of the system is:

$$E_{ss} = \frac{fR}{f\frac{V_{max}}{K_m} + u}. \quad (5)$$

The coefficient u is very small because most released dopamine is taken back up by the terminals (though not necessarily into the same terminal that released it) [17,18]. Because u is small, the graph of E_{ss}, given by Eq. (5), remains very flat as f declines from $f = 1$ and plunges toward zero only when f gets quite small. This explains why the passive stabilization effect fails when f gets small and is the underlying reason why the solid curves in Figs. 1–3 have the shapes that they have.

In fact, we believe, as explained above, that the removal coefficient itself gets bigger as f gets smaller. To experiment realistically with this complication and to include the highly non-linear effects of the autoreceptors on synthesis it is necessary to use the full detailed model in [14] as we have done above.

Discussion

Our purpose is to clarify the competing effects that influence the stabilization of EDA even in the face of substantial cell loss in the SNc. The basic reason for the homeostasis of EDA is Garris' passive stabilization effect that we show depends on the number of DATs. This passive stabilization effect disappears when f (fraction of remaining terminals) is small due to removal of dopamine from the system; the removal increases as f declines, compromising the homeostatic effect. Finally, once EDA begins to drop, the inhibition of tyrosine hydroxylase by the autoreceptors gradually is relieved, so synthesis is increased and EDA is further stabilized. The resulting curves (solid lines in Figs. 2 and 3) reflect a balance of all three mechanisms, passive stabilization, removal from the system, and the release of inhibition by the autoreceptors. For moderate lesions ($0.25 \leq f \leq 1$), it is the passive stabilization mechanism proposed by Garris that creates EDA homeostasis. For more severe lesions ($f < 0.25$), the upregulation of synthesis and release (via the autoreceptors) plays a major homeostatic role.

These hypotheses can be tested experimentally in various ways. Caron and co-workers have developed a DAT knockout mouse [17]. If one measured the striatal EDA steady state concentration in wild-type, DAT knockout heterozygote, and the DAT knockout homozygote, at different levels of denervation (different f), one should obtain three quite different curves. For the wild-type, the EDA concentration should be very stable as f decreases as in the solid curve in Fig. 1. For the heterozygote (50% of the normal amount of DATs), one should get a curve between the solid curve and the 25% curve in Fig. 1. And for the homozygote, the EDA curve should descend linearly to zero as f goes from 1 to 0. The DATtg mouse [27] overexpresses the DAT transporter by 30%, so the same experiments in that mouse should give a curve above (i.e. more homeostatic) the curve for wild-type in Fig. 1. This would confirm Garris' hypothesis and our calculations that suggest that for moderate lesions the stabilization of EDA is produced by the DATs. Next, a number of DA auto-receptor antagonists are known, such as (−)-sulpiride and domperidone. If the same experiments are carried out in the presence of autoreceptor antagonists, all of the curves discussed above should move down (less homeostasis), but the effects should not be significant until f is small (large lesion). This would confirm Garris' suggestion and our calculations that suggest that the autoreceptors play an important role in stabilizing EDA only when lesions are quite large.

It very interesting to speculate whether a similar passive stabilization of extracellular serotonin is caused by the serotonin reuptake transporters. The synthesis and reuptake mechanisms for serotonin are quite similar to those for dopamine, so one would expect similar overall behavior in serotonergic systems. We are currently constructing a mathematical model for a serotonergic neuron to investigate this question. Unfortunately, the level of extracellular serotonin is very low in the striatum [28] and the frontal cortex [29] and the serotonergic projections come from various, small nuclei [1], so an exact measure of extracellular serotonin as a function of denervation will be difficult. However, such measurements may be very important for understanding the effects of selective serotonin reuptake inhibitors and designing treatments for clinically depressed patients.

Conflict of interest

The authors declare that they have no financial or commercial conflicts of interest.

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